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Charlie Auer
Director
TSCA Document Control Office (7407)
EPA East Building, Room 6428
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
1201 Constitution Avenue, NW
Washington, DC 20460-0001

Dear Mr. Auer:

The American Chemistry Council makes available to the public and appropriate government agencies final reports of environmental, health, and safety research that it sponsors. In keeping with this policy, the following reports that the American Chemistry Council Ethylbenzene and Olefins Panel recently conducted are enclosed:

"A Pilot Inhalation Study for a Reproductive Toxicity Study of Ethylbenzene in Rats," and

"1-Butene: Combined Repeated-Exposure Toxicity, Reproduction and Neurotoxicity Screening in Rats Via Whole-Body Inhalation Exposures.

The enclosed reports do not include confidential information.

If you have any questions, please call the Ethylbenzene and Olefins Panel Manager of my staff, Dr. Elizabeth Moran, at 301-924-2006.

Sincerely yours,

Marian K. Stanley for CMP

Enclosure

Contain NO CBI

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FINAL REPORT

Volume 1 of 4
(Text and Summary Tables 1-104)

STUDY TITLE

A PILOT INHALATION STUDY FOR A REPRODUCTIVE TOXICITY STUDY OF ETHYLBENZENE IN RATS

STUDY NUMBER

WIL-186028

STUDY DIRECTOR

Donald G. Stump, Ph.D., D.A.B.T.

STUDY INITIATION DATE

August 27, 2002

STUDY COMPLETION DATE

August 1, 2003

PERFORMING LABORATORY

WIL Research Laboratories, Inc.
1407 George Road
Ashland, OH 44805-9281

AMERICAN CHEMISTRY COUNCIL CONTRACT NUMBER

EB-13.0-VCCEP-Research-WIL

SPONSOR

American Chemistry Council
Ethylbenzene Panel
1300 Wilson Boulevard
Arlington, VA 22209

1. SUMMARY

1.1. OBJECTIVE

This study was conducted to determine the exposure levels of the test article, ethylbenzene, that will generate minimal maternal and weanling toxicity but allow complete gestation, parturition and successful lactation, to determine the feasibility of gavage dosing of dams during lactation days 1-4, and to determine whether to begin inhalation exposures of F_1 weanlings on PND 22 or PND 29 in a subsequent two-generation reproductive toxicity study.

1.2. STUDY DESIGN

Four groups of male and female Crl:CD®(SD)IGS BR rats (20/sex/group) were exposed to either clean filtered air or vapor atmospheres of the test article. Target test article concentrations were 100, 500 and 1000 ppm (parts per million) for the F_0 generation and selected F_1 weanlings. Mean measured inhalation exposure concentrations were 99, 500 and 1008 ppm for the F_0 generation and 101, 498 and 1002 ppm for the F_1 generation. F_0 males were exposed to the test atmospheres for a minimum of four weeks. F_0 females were exposed to the test atmospheres for a minimum of two weeks prior to mating, throughout the mating period and from gestation day 0 through gestation day 20. At this time, one-half of the F_0 females were selected for the inhalation/gavage phase and the remaining females were selected for the inhalation only phase. Inhalation exposure of the F_0 females in both phases was suspended from gestation day 21 through lactation day 4. In the inhalation/gavage phase, F_0 females received the vehicle, corn oil, or the test article in the vehicle via oral gavage at dose levels of 0, 90, 342 and 621 mg/kg/day (divided into three equal doses, approximately two hours apart) at a dose volume of 1 mL/kg/dose during lactation days 1 through 4. Inhalation exposure of the F_0 females in both phases was re-initiated on lactation day 5 and continued through the day prior to euthanasia. The F_1 offspring were potentially exposed to the test article *in utero* (placental transfer), through nursing during lactation until weaning. On lactation days 21 and 28, pups were weaned and selected (one pup/sex/litter) for exposure to the same concentration of the

test article as their dam beginning on PND 22 or 29 and lasting through PND 33. Pups not selected for post-weaning exposures were necropsied following weaning on PND 28. For reporting purposes, the F_0 male exposure group designations were 100 ppm, 500 ppm and 1000 ppm. Exposure group designations for all F_0 females through the pre-mating and gestation periods were 100 ppm, 500 ppm and 1000 ppm. Exposure group designations for F_0 females that also received gavage doses of the test article and for all the F_1 offspring of these dams were 100 ppm/90 mg/kg, 500 ppm/342 mg/kg and 1000 ppm/621 mg/kg.

All animals were observed twice daily for moribundity and mortality, appearance, behavior and pharmacotoxic signs (prior to inhalation exposure and/or gavage dosing, at the midpoint of inhalation exposure and approximately one hour after completion of administration of the test article). Clinical observations, body weights and food consumption were recorded at appropriate intervals prior to mating and during gestation and lactation. All F_0 and F_1 females were allowed to deliver and rear their pups until weaning on lactation day 28, except for one pup/sex/litter that was weaned on PND 21. Ten pups per litter (of equal sex distribution, if possible) were selected on PND 4 to reduce the variability among the litters. Pups were observed daily for general appearance and behavior, and survival. Detailed physical examinations and body weights were recorded at appropriate intervals.

All F_0 males were necropsied following the breeding period. F_0 females were necropsied on lactation day 28; F_0 females which failed to deliver were necropsied on post-mating day 25 (females with evidence of mating) or post-cohabitation day 25 (females without evidence of mating) and F_0 females with total litter losses were euthanized within 24 hours of litter loss. Selected organs were weighed. All surviving pups were euthanized on PND 28 or 34 and received a gross external exam prior to being discarded.

1.3. RESULTS

No adverse exposure-related survival, clinical signs or macroscopic findings were noted at any exposure level in the F_0 generation. Increased mean liver (males and females) and kidney (males only) weights were observed in the 500 and 1000 ppm groups.

Body weight gain was decreased in the 500 and 1000 ppm group males and females during the first week of exposure and continued to be reduced in the 1000 ppm group males during the second week of exposure. Body weight parameters were unaffected in the low exposure group (100 ppm or 100 ppm/90 mg/kg).

There were no indications of adverse effects on reproductive performance in the F_0 generation. Male and female mating and fertility indices, pre-coital intervals, gestation lengths and live litter size were similar at all exposure levels. Postnatal survival was slightly reduced from birth to PND 4 in the 500 and 1000 ppm groups (inhalation phase) and in the 1000 ppm/621 mg/kg group (inhalation/gavage phase) as a result of large numbers of pup loss in single litters. Mean pup weights (male and female) in the 1000 ppm group were significantly decreased on PND 1 (11.1% and 9.0%, respectively). These reductions continued throughout the pre-weaning period in the 1000 ppm/621 mg/kg group (inhalation/gavage phase). In the inhalation phase, mean male pup body weights in the 500 and 1000 ppm groups were slightly lower than the control group on PND 14 and 21 (9.2-10.9% and 8.4-12.6%, respectively). Mean male and female offspring body weights in the 100 and 500 ppm groups for both the inhalation and inhalation/gavage phases were similar to the control group on PND 1.

Several exposure-related deaths were observed in the 500 and 1000 ppm group (inhalation phase) weanlings and the 1000 ppm/621 mg/kg group (inhalation/gavage phase) weanlings that initiated exposure on PND 22. Exposure-related clinical signs observed one hour following dosing included labored respiration, eyelids half-closed, prostrate, animal unable to right itself, and rocking, lurching and swaying while ambulating. Mean body weight gain was reduced in the F_1 weanlings exposed to 500 and 1000 ppm beginning on PND 22 in both the inhalation and inhalation/gavage phases.

Slightly reduced mean body weights were noted for the F_1 weanlings exposed to 100 ppm beginning on PND 22 in both the inhalation and inhalation/gavage phases.

No deaths or exposure-related clinical signs were noted in the F_1 weanlings exposed to the test article beginning on PND 29. Mean body weight gain was reduced in these weanlings exposed to 500 or 1000 ppm beginning on PND 29 in both the inhalation and inhalation/gavage phases.

No adverse effects were observed on clinical signs or body weights in the F_1 weanlings exposed to 100 ppm of test article beginning on PND 22 or 29.

1.4. CONCLUSIONS

Based on the results of this study, gavage dosing of dams on lactation days 1-4 will be performed and weanling inhalation exposure will initiate on PND 22 in a definitive two-generation reproductive toxicity study of ethylbenzene via whole-body inhalation. Exposure levels for this study will be 25, 100 and 500 ppm, and gavage dose levels on lactation days 1-4 will be 26, 90 and 342 mg/kg/day.

STUDY NO. 02-4224
SPONSOR STUDY NO. OLF–83.0–HPV2–HLS

1-BUTENE:

COMBINED REPEATED-EXPOSURE TOXICITY, REPRODUCTION AND NEUROTOXICITY SCREENING IN RATS VIA WHOLE-BODY INHALATION EXPOSURES

Final Report

Volume I of II

Performed by: Huntingdon Life Sciences
Mettlers Road
East Millstone, New Jersey 08875-2360

Submitted to: American Chemistry Council
1300 Wilson Boulevard
Arlington, VA 22209

Attn: Elizabeth J. Moran, Ph.D., D.A.B.T.

Date: 01 August 2003

SUMMARY

This study was designed to assess the potential toxicity, including neurotoxicity and reproductive performance, in male and female rats when 1-Butene was administered as a gas by whole-body inhalation exposures. Reproductive effects were assessed by histological examination of the reproductive organs, mating behavior, conception, development of the conceptus, parturition, and pup survival to Lactation Day 4.

The test substance was administered to Sprague Dawley rats (12/sex/Main Study group and 12 females/Satellite group) at target concentrations (based on results of prior testing with similar chemicals and the lower explosion limits for the test article) of 500, 2000 and 8000 ppm for 6 hours/day, 7 days/week for 2 weeks before mating intitiation. Main Study females were for subchronic evaluations and Satellite females were for reproductive evaluations only. Exposure of Main Study males (12/group) continued for a minimum exposure of 28 days (during mating and post-mating until they were euthanized), while Main Study females (12/group) were exposed once daily (6 hours/day), 7 days/week for 28 days. Satellite females (12/group) continued to be treated once daily (6 hours/day) during mating, and then once daily (6 hours/day) during gestation (Days 0-19). Those Satellite animals without evidence of mating (but which were actually pregnant) continued treatment (6 hours/day) following completion of the mating period until their estimated Gestation Day 19. In addition, a control group (12 males and 24 females) received nitrogen enriched air only while in chamber. Exposure levels were determined using an on-line gas chrotomatograph 4 times per chamber per day. Particle size distribution measurements were also made once per chamber per week using a TSI Aerodynamic Particle Sizer.

Viability checks were performed twice daily to check for mortality and signs of severe toxic or pharmacologic effects. Physical observations and body weight measurements were made once pretest and at least weekly during the study. Animals were also observed once during each exposure. Satellite female rats had a detailed physical observation performed weekly during the premating period and on Gestation Days 0, 7, 14, 20 and Lactation Days 0, 1 and 4. Mated Satellite female rats were weighed on Gestation Days 0, 7, 14 and 20 and Satellite female rats that delivered litters were weighed on Lactation Days 1 and 4. Feed consumption measurements were obtained beginning the week prior to treatment initiation and at least weekly during the study with the exception of during the mating period for males and Satellite females. For pregnant or confirmed mated Satellite female rats, feed consumption was recorded on Gestation Days 0-7, 7-14, and 14-20 and on Lactation Days 1-4. Neurobehavioral (Functional Observational Battery and Locomotor Activity) examinations were performed at pretest and during the last week of exposure (on a non-exposure day) on all Main Study male rats and all Main Study female rats from all exposures. Hematology, coagulation, and clinical chemistry were performed

SUMMARY

on 12 animals/sex/main study group at study termination. After completion of exposures, all parental animals (P_0 generation: Main Study males and Satellite females) were sacrificed. Selected organs were weighed and organ/body weight and organ/brain weight ratios calculated. Complete macroscopic postmortem examinations were performed on all parental animals. Histopathological evaluations of selected tissues were conducted on selected parental animals.

Pups (F_1 generation) were observed as soon as possible after parturition for their sex, the number of live and dead pups and pup abnormalities. Thereafter, litters were observed twice daily (morning and afternoon) and gross physical examinations were performed on Lactation Days 0 and 4. Pups were sexed on Lactation Day 0 and sex verified on Lactation Day 4. Individual pup body weights were recorded on Lactation Days 1 and 4. Pups surviving until Lactation Day 4 were euthanized followed by a macroscopic postmortem examination (external), in which any unusual abnormalities were noted and then the carcasses were discarded.

The test article, purchased from MG Industries, was assayed by GC versus an analytical standard, purchased from Aldrich Chemicals, before and after the study to demonstrate the purity and stability of the test article. The test article was determined to be 100% 1-Butene before the study and 99.84% 1-Butene (with the balance Isobutane) after the study demonstrating the purity and the stability of the test article. Chamber distribution analyses showed that the test article was evenly distributed within each chamber. The mean (± standard deviation) analytical (GC) concentrations for the control and the exposure groups were as follows: 0 ± 0, 524 ± 40, 2062 ± 126 and 8271 ± 683. The analytically measured exposure levels of the airborne test article were reasonably close to the targeted exposure levels. Chamber environmental conditions averaged 23°C temperature and 57% relative humidity. Mean particle size distribution measurements for the exposures indicated that the atmospheres were gas only, as expected, since there was no substantial difference between the test article chambers and the air control chambers.

There was no effect of treatment on survival. All animals survived until the termination of the study. The test animals were unremarkable during the exposure periods (in-chamber) and during the non-exposure periods. There were no exposure-related differences in body weights or weight changes or feed consumption in the test article exposed animals compared to the Air Control animals. There was no apparent exposure-related effect on motor activity or functional observational battery parameters for either sex in this study. There were no exposure-related differences in hematology or coagulation values or clinical chemistry values in test article exposed animals compared to the Air Control animals at the terminal interval.

SUMMARY

There were no exposure-related differences in macroscopic postmortem evaluations or organ weights in the test article exposed animals compared to the Air Control animals. There were no microscopic findings considered to be related to exposure to 1-Butene.

All mated female animals (except one animal in the 2000 ppm group) were found pregnant and delivered live pups. Mating indices for the male rats treated with the test article were comparable to the Air Control group. Mating, fertility and gestation indices for the female rats treated with the test article were comparable to the Air Control group. Most of the females in each group mated at the first opportunity. There were also no treatment-related differences in the other reproductive parameters up to the time of parturition including the percent of females completing delivery and the duration of gestation, when compared to the Air Control group. There were no treatment-related differences in all parturition parameters including the total number of pups delivered, the number of pups dying, the viability (4 day survival) index, the number of implantation sites and corpora lutea per dam, the pup sex ratio and the number of live pups/litter, when compared to the Air Control group. The pups were unremarkable during the lactation period. There were no exposure-related differences in body weights or weight gains in the pups feeding from test article exposed animals compared to the pups feeding from Air Control animals. There were no exposure-related differences in macroscopic postmortem evaluations in the pups feeding from test article exposed animals compared to the pups feeding from Air Control animals.

In conclusion, exposure of male and female rats to target concentrations of 500, 2000 and 8000 ppm of 1-Butene resulted in no general systemic effects or effects on reproductive performance. Therefore, a no observed effect level (NOEL) of 8000 ppm was determined.